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     FILE 'MEDLINE' ENTERED AT 12:52:03 ON 21 JAN 2005
L1            14 S 293EBNA
L2             0 S L1 AND GENE THERAPY
L3             8 S L1 AND GENE
L4             0 S L1 AND TRANSGENIC
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L3 ANSWER 1 OF 8 MEDLINE on STN
TI Antisense overexpression of BMAL2 enhances cell proliferation.
PY 2003
AU Yeh Chau-Ting; Lu Su-Chuan; Tseng I-Chu; Lai Hsin-Yu; Tsao Mei-Lin; Huang Shiu-Feng; Liaw Yun-Fan
AB To identify **genes** that are frequently downregulated in hepatocellular carcinoma (HCC), a panel of putative underexpressed **genes** was first established by an in-house cDNA macroarray method. Two different assays, semiquantitative RT-PCR combined with Northern analysis and customized cDNA microarray analysis, were used to screen through these **genes** and the results were compared. Several **genes**, some with unknown function, were confirmed to be downregulated by both the methods. The effect of a downregulated **gene**, BMAL2, on cell proliferation was examined. Overexpression of antisense BMAL2 RNA in **293EBNA** cells resulted in reduced cell cycle time, increased plating efficiency in soft agar, diminished TNF-alpha-induced increment of CPP32/caspase-3 activity, and a reduced proportion of cells in the G2 phase with a concomitantly increased proportion of cells in the S phase. In conclusion, by combining three different methods, we have obtained a panel of frequently down regulated **genes** in HCC, including BMAL2. Antisense overexpression of BMAL2 enhances cell proliferation.

L3 ANSWER 2 OF 8 MEDLINE on STN
TI The N-terminal internal region of BLM is required for the formation of dots/rod-like structures which are associated with SUMO-1.
PY 2001
AU Suzuki H; Seki M; Kobayashi T; Kawabe Yi; Kaneko H; Kondo N; Harata M; Mizuno S; Masuko T; Enomoto T
AB Bloom Syndrome (BS) is a human autosomal genetic disorder characterized by a predisposition to a variety of malignant tumors. The **gene** responsible for BS encodes a protein (BLM) consisting of 1417 amino acids with a nuclear localization signal in the C-terminal region, which is a member of the RecQ helicase family. We previously showed, using a yeast two-hybrid system, that BLM interacted with Ubc9, which is the conjugating enzyme of SUMO-1 (small ubiquitin-related modifier-1). In the present study, we exogenously expressed a green fluorescent protein-tagged Bloom syndrome protein, GFP-BLM, in human **293EBNA** cells and found that it formed dots/rod-like structures associated with SUMO-1 in the nucleus. Deletion experiments indicated that the region from amino acids 238 to 586 of BLM is required for the formation of dots/rod-like structures associated with SUMO-1, and the DNA helicase domain, but not the helicase activity itself, slightly affected the formation and/or stability of these structures. Expression of a GFP-BLM which contained the 238-586 region, but lacked the C-terminal nuclear localization signal, resulted in localization to the cytoplasm without the formation of dots/rod-like structures and association with SUMO-1, indicating that these events occur only in the nucleus.

L3 ANSWER 3 OF 8 MEDLINE on STN
TI The human homologue of the yeast mitochondrial AAA metalloprotease Yme1p complements a yeast yme1 disruptant.
PY 2000
AU Shah Z H; Hakkaart G A; Arku B; de Jong L; van der Spek H; Grivell L A; Jacobs H T
AB In yeast, three AAA superfamily metalloproteases (Yme1p, Afg3p and Rca1p) are localized to the mitochondrial inner membrane where they perform roles in the assembly and turnover of the respiratory chain complexes. We have investigated the function of the proposed human orthologue of yeast Yme1p, encoded by the YME1L **gene** on chromosome 10p. Transfection of

both HEK-**293EBNA** and yeast cells with a green fluorescent protein-tagged YME1L cDNA confirmed mitochondrial targeting. When expressed in a yme1 disruptant yeast strain, YME1L restored growth on glycerol at 37 degrees C. We propose that YME1L plays a phylogenetically conserved role in mitochondrial protein metabolism and could be involved in mitochondrial pathologies.

L3 ANSWER 4 OF 8 MEDLINE on STN
TI Human RecQ5beta, a large isomer of RecQ5 DNA helicase, localizes in the nucleoplasm and interacts with topoisomerases 3alpha and 3beta.
PY 2000
AU Shimamoto A; Nishikawa K; Kitao S; Furuichi Y
AB The RecQ helicase superfamily has been implicated in DNA repair and recombination. At least five human RecQ-related **genes** exist: RecQ1, BLM, WRN, RecQ4 and RecQ5. Mutations in BLM, WRN and RecQ4 are associated with Bloom, Werner and Rothmund-Thomson syndromes, respectively, involving a predisposition to malignancies and a cellular phenotype that includes increased chromosome instability. RecQ5 is small, containing only a core part of the RecQ helicase, but three isomer transcripts code for small RecQ5alpha (corresponding to the original RecQ5 with 410 amino acids), new large RecQ5beta (991 amino acids) and small RecQ5gamma (435 amino acids) proteins that contain the core helicase motifs. By determining the genomic structure, we found that the three isoforms are generated by differential splicing from the RecQ5 **gene** that contains at least 19 exons. Northern blot analysis using a RecQ5beta-specific probe indicates that RecQ5beta mRNA is expressed strongly in the testis. Immunocytochemical staining of three N-terminally tagged RecQ5 isomers expressed in **293EBNA** cells showed that RecQ5beta migrates to the nucleus and exists exclusively in the nucleoplasm, while the small RecQ5alpha and RecQ5gamma proteins stay in the cytoplasm. Immunoprecipitation and an extended cytochemical experiment suggested that the nucleoplasmic RecQ5beta, like yeast Sgs1 DNA helicase, binds to topoisomerases 3alpha and 3beta, but not to topoisomerase 1. These results predict that RecQ5beta may have an important role in DNA metabolism and may also be related to a distinct genetic disease.

L3 ANSWER 5 OF 8 MEDLINE on STN
TI Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers.
PY 1999
AU Stacker S A; Stenvers K; Caesar C; Vitali A; Domagala T; Nice E; Roufail S; Simpson R J; Moritz R; Karpanen T; Alitalo K; Achen M G
AB Vascular endothelial growth factor-D (VEGF-D) binds and activates the endothelial cell tyrosine kinase receptors VEGF receptor-2 (VEGFR-2) and VEGF receptor-3 (VEGFR-3), is mitogenic for endothelial cells, and shares structural homology and receptor specificity with VEGF-C. The primary translation product of VEGF-D has long N- and C-terminal polypeptide extensions in addition to a central VEGF homology domain (VHD). The VHD of VEGF-D is sufficient to bind and activate VEGFR-2 and VEGFR-3. Here we report that VEGF-D is proteolytically processed to release the VHD. Studies in **293EBNA** cells demonstrated that VEGF-D undergoes N- and C-terminal cleavage events to produce numerous secreted polypeptides including a fully processed form of M(r) approximately 21,000 consisting only of the VHD, which is predominantly a non-covalent dimer. Biosensor analysis demonstrated that the VHD has approximately 290- and approximately 40-fold greater affinity for VEGFR-2 and VEGFR-3, respectively, compared with unprocessed VEGF-D. In situ hybridization demonstrated that embryonic lung is a major site of expression of the VEGF-D **gene**. Processed forms of VEGF-D were detected in embryonic lung indicating that VEGF-D is proteolytically processed in vivo.

L3 ANSWER 6 OF 8 MEDLINE on STN
TI Rapid generation of stable cell lines expressing corticotropin-releasing hormone receptor for drug discovery.
PY 1997
AU Horlick R A; Sperle K; Breth L A; Reid C C; Shen E S; Robbins A K; Cooke G M; Largent B L
AB Human HEK293 cells that stably express the Epstein Barr nuclear antigen 1 (EBNA1) support the episomal replication of plasmids containing the Epstein Barr virus origin of replication (EBV oriP). A **293EBNA** (293E) cell line expressing the human corticotropin-releasing hormone receptor subtype I (CRHR1) from an episomal plasmid was generated (293CR1s), analyzed, adapted to spinner culture, and scaled-up for production in less than 6 weeks. Forty-seven stable CHO cell lines transfected with CRHR1 were also isolated. Expression of the receptor in the best of these lines (as judged by CRH-induced cAMP production), CHO-R22, was compared to that in 293CR1s cells. Results indicate that the CRHR1 episomal expression vector in 293E cells (1) rapidly generates stable cell lines suitable for scale-up; (2) is stably maintained during 3 months in culture; (3) expresses high levels of CRHR1 mRNA; and (4) expresses significantly more CRHR1 than the CHO-R22 line. Coexpression of additional G protein alpha subunit (G alpha s) with CRHR1 in 293E cells converts a higher percentage of receptor to the agonist high-affinity G-protein-coupled state. Our data support the idea that using the EBV oriP-driven episomal system for **gene** expression results in greater production of protein in a relatively short period of time.

L3 ANSWER 7 OF 8 MEDLINE on STN
TI Vascular endothelial growth factor B, a novel growth factor for endothelial cells.
PY 1996
AU Olofsson B; Pajusola K; Kaipainen A; von Euler G; Joukov V; Saksela O; Orpana A; Pettersson R F; Alitalo K; Eriksson U
AB We have isolated and characterized a novel growth factor for endothelial cells, vascular endothelial growth factor B (VEGF-B), with structural similarities to vascular endothelial growth factor (VEGF) and placenta growth factor. VEGF-B was particularly abundant in heart and skeletal muscle and was coexpressed with VEGF in these and other tissues. VEGF-B formed cell-surface-associated disulfide-linked homodimers and heterodimerized with VEGF when coexpressed. Conditioned medium from transfected **293EBNA** cells expressing VEGF-B stimulated DNA synthesis in endothelial cells. Our results suggest that VEGF-B has a role in angiogenesis and endothelial cell growth, particularly in muscle.

L3 ANSWER 8 OF 8 MEDLINE on STN
TI Improved expression cloning using reporter **genes** and Epstein-Barr virus ori-containing vectors.
PY 1995
AU Shen E S; Cooke G M; Horlick R A
AB Levels of expression of two reporter **genes** cloned into SV40 or Epstein-Barr virus (EBV) ori-containing plasmids were measured following transient transfection of cell lines constitutively expressing T-antigen or EBV nuclear antigen 1 (EBNA1). The TSA201 and COS7 cell lines stably produce T-antigen and support replication of the SV40 ori-containing constructs while the **293EBNA** cell line produces EBNA1 and supports replication of EBV ori-containing plasmids. We found that **293EBNA** cells express > 25-fold more beta-galactosidase (beta Gal) per mg protein than COS7 cells and 11-fold more beta Gal than TSA201 cells. We also demonstrate that **293EBNA** cells are able to express 70-100-fold more angiotensin II type-1 receptor (AT1) per mg protein than COS7 or TSA201 cells. We examined the suitability of each cell line for use in expression cloning using a NaOH 'scrape' method as an

improvement over emulsion autoradiography for detection. Measurable AT1 signals can be detected when reporter plasmids are diluted up to 1000-fold for COS7 and TSA201 cells, and up to 80,000-fold for **293EBNA** cells. These data demonstrate that **293EBNA** cells offer a significant improvement in expression cloning technology as compared to the conventionally used T-antigen-based cell lines.